Water Spray and Immersion in Chemical Sanitizer to Lower Bacterial Numbers on Broiler Transport Coop Flooring[1]

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Primary Audience: Researchers, Plant Managers, Live Production Managers

SUMMARY

Broiler transport coops become soiled with feces during use. When this fecal matter contains *Campylobacter*, the result can be cross-contamination of subsequent flocks that were previously free of this important human pathogen. Because washing and sanitizing coops requires large amounts of water and is not always effective enough to justify the expense, few broiler companies wash and sanitize dump coops between flocks. In this lab-scale study, a tap water spray was effective in lowering the numbers of bacteria, including *Campylobacter*, associated with broiler transport coop flooring. Immersion in a chemical sanitizer after spray washing did not enhance the antibacterial effect. It is possible that sanitizing treatments could be made more effective by using higher concentrations of chemicals, high temperature treatment, or high pressure or repeated applications of water spray. However, such changes would come at a cost. Research is needed to find new and innovative ways to lower bacterial numbers in broiler transport coops without undue use of water and the associated expense.

Key words: broiler, *Campylobacter*, *Escherichia coli*, sanitize, transport cage, transport coop

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DESCRIPTION OF PROBLEM

Campylobacter is an important human pathogen causing many cases of foodborne illness in recent years. Numerous studies have linked *Campylobacter* and campylobacteriosis to poultry and poultry meat products [1, 2, 3]. Therefore, it is important to the poultry industry and poultry consumers that researchers strive to understand microbial ecology as it relates to this organism. Such information will provide insight into the best possible means to lower the prevalence and numbers of *Campylobacter* on poultry products.

During the course of catching, transport, and holding at the plant, broilers can spend from 3 to 12 h in transport coops. While held in these coops, broilers continue to defecate, resulting in soiled coop surfaces. This is especially problematic because feces expressed during this time may be more heavily laden

[1]Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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with *Campylobacter*. During feed withdrawal, the microflora in the alimentary tract changes, and the pH of crop contents increases from acidic to nearly neutral [4]. This results in a more favorable environment for *Campylobacter* [5]. Following feed withdrawal, the stressors associated with transport have been shown to increase the numbers of *Campylobacter* expressed from the cloacae of broilers upon arrival at the plant and during holding [6]. Therefore, it is little wonder that *Campylobacter* can be recovered from transport coops [7, 8, 9].

It has been suggested [7, 9], and we have since confirmed [10], that *Campylobacter* left in soiled transport coops can lead to the contamination of a subsequent load of broilers placed in those coops. Contamination acquired from the transport coop has been shown to persist on the carcass after scalding and defeathering [10].

Decontamination of transport coops and crates has been studied. Some techniques tested include immersion of an entire 5-tier coop into a tank of heated or unheated sanitizer solution [11, 12, 13]. These methods seem to be effective in studies that are small in scale relative to the US poultry industry. However, they have not been proven in commercial settings. Other reports show limited effectiveness of washing and sanitizing procedures against bacteria attached to steel surfaces [14, 15]. Although these surfaces are important, much of the fecal matter in transport coops is located on the floor, which is typically made of fiberglass.

When coop and crate washing techniques used in commercial settings were closely examined, the procedures were not always successful. There have been several studies that show despite careful cleaning and sanitizing, including removal of visible feces, *Campylobacter* can still be recovered from crates and coops that have been commercially washed in preparation for receiving broilers [8, 16, 17]. One study [17] documented that the numbers remaining in the coop were high enough to result in intestinal colonization of birds that were placed therein.

A large amount of water is being used in the US for commercial broiler processing. The average processing plant uses approximately 7 gal of water for each bird processed [18]. This is an economic and environmental problem because of the limited availability of fresh water for local communities, high water prices, and strict wastewater discharge requirements. If coop washing procedures are not adequate to remove contamination, then one must consider whether the effort is worth the expense both environmentally and economically.

The objective of this study was to examine the effectiveness of a tap water spray with and without an immersion dip in a chemical sanitizer as an intervention technique applied to soiled transport coop flooring. The approach was to apply the treatments to fiberglass transport coop flooring material that had been intentionally contaminated with broiler gut contents containing *Campylobacter*.

MATERIALS AND METHODS

Experimental Overview and Design

Squares of fiberglass flooring material (5 × 5 cm) were used as a model of broiler transport coop floors. One gram of gut contents was spread evenly on each square to simulate fecal contamination excreted by birds during transport and holding. After a 60-min drying period, a tap water spray was used to wash gut contents from the flooring followed by immersion in 1 of 2 sanitizers. Untreated controls and water sprayed/unsanitized controls were included for comparison. Three sets of experiments were conducted, each with a different length of time for sanitizer immersion. For each set of experiments, 3 replications were conducted, each with 10 squares of flooring per treatment.

Flooring

Used fiberglass transport coop flooring was donated by a commercial broiler company. Flooring material was thoroughly scrubbed and cut into 5×5 cm squares (25 cm^2). Prior to each experiment, flooring squares were sterilized by autoclaving at 121°C for 15 min. Floor squares were held aseptically in a covered beaker until use.

Contamination with Gut Contents

A number of intestinal tracts were collected from the evisceration line in a commercial

broiler processing plant on the day of each replication. The contents of each intestinal tract (small intestine, cecum, and colon) were removed by manual expression and combined in a sterile specimen cup.

Campylobacter was our primary organism of interest; to assure its presence we inoculated gut contents with a field strain of *Campylobacter* originally isolated from naturally contaminated broiler gut contents. To make the inoculum, the field strain was grown overnight on Campy-cefex agar (CCA) [19, 20]. A number of *Campylobacter* colonies were removed from the agar surface and suspended in PBS to result in a cell suspension with an optical density of 0.350 at a wavelength of 540 nm [21]. Ten microliters of cell suspension was added per gram of gut contents, resulting in approximately 10^7 cells/g for application to each square of floor material.

Following inoculation, gut contents were thoroughly mixed with a sterile instrument; 1 g was placed on each fiberglass square and spread evenly with a laboratory spatula. Gut contents were allowed to remain on the flooring material for 60 min at room temperature (average temperature was 24°C) before washing.

Water Spray Treatment

Tap water (average total chlorine of 0.5 ppm) was used to spray gut contents off of the fiberglass squares. Water was applied as a spray from a laboratory sink faucet fitted with a nozzle (4 mm internal diameter) and a pressure gauge. Water pressure was set at 10 psi, and the flow rate was 1,500 mL/15 s. Each square was held 6 to 8 cm from the nozzle tip and moved back and forth under the water spray for 15 s to allow maximum removal of visible gut contents. The 10 squares per treatment type were sprayed with water, one at a time. The sink used for inoculated replications was located in a biocontainment building such that all spray water was subjected to a bactericidal heat treatment prior to release into a municipal sewer.

Sanitizer Treatments

Flooring squares that were to be treated with sanitizer were staged until all 10 had been sprayed with water. Ten squares per treatment were immersed together in a presterilized plastic pan containing 500 mL of sanitizer compound. Two sanitizers were tested, a quaternary ammonium chloride compound [22] and sodium hypochlorite [23]. Both sanitizers were prepared such that the concentration of active ingredient was 200 ppm (as suggested by the manufacturer of the quaternary ammonium compound). Total chlorine concentration was confirmed by testing with a chlorine meter [24].

In the first set of experiments the sanitizer immersion treatment was 15 s, in the second set washed squares were immersed for 60 s, and in the third set squares were exposed to the sanitizer liquid for 5 min. After being sanitized, floor squares were placed in a sterile rack and allowed to drip dry at an angle of approximately 45° for 15 min prior to sampling.

Bacterial Culture

Following treatment with water wash, sanitizer, and drying, each floor square was sampled with separate, premoistened sterile cotton tipped applicators. The cotton tip of the applicator was pressed to the surface and manually moved back and forth to cover the entire square. The flooring square was then rotated 90°, and the swab was rubbed back and forth over the surface again.

All swabs were premoistened by dipping in 10 mL of D/E neutralizing broth [25]. This broth counteracts the bacterial inhibition affect of the sanitizing chemicals stopping any reaction at the time of sampling. After sampling, each swab was replaced into 10 mL of D/E neutralizing broth. Serial dilutions from each tube with a sample swab were made in PBS and used to plate onto the surface of Campy-cefex agar and Petrifilm *E. coli*/coliform count plates [26]. Campy cefex plates were incubated [20], and colonies characteristic of *Campylobacter* were counted. Suspect colonies were confirmed as *Campylobacter* by observation of cellular motility and morphology under phase contrast microscopy and serological latex agglutination test [27]. Petrifilm *E. coli*/coliform count plates were incubated [28]; coliform and *E. coli* colonies were counted as specified in the instructions. All bacterial counts were transformed to $\log_{10}$ colony-forming units re-

TABLE 1. Mean number of bacteria (log cfu ± 95% confidence interval) recovered from squares (5 × 5 cm) of fiberglass transport coop flooring originally contaminated with 1 g of gut contents then subjected to a 15-s spray of water and a 15-s dip in sanitizer (n = 30)

Treatment	*Campylobacter*	Coliforms	*Escherichia coli*
None (control)	$7.1^{a} \pm 0.2$	$6.3^{a} \pm 0.1$	$6.0^{a} \pm 0.1$
Water wash[1] only	$5.1^{bc} \pm 0.3$	$4.8^{b} \pm 0.1$	$4.4^{b} \pm 0.1$
Water wash + sanitizer[2]	$5.2^{b} \pm 0.3$	$4.9^{b} \pm 0.1$	$4.4^{b} \pm 0.1$
Water wash + chlorine[3]	$4.9^{c} \pm 0.3$	$4.7^{b} \pm 0.1$	$4.3^{b} \pm 0.1$

[a–c]Values within columns with different superscripts are significantly different by Tukey's honestly significant difference (HSD) at $P < 0.05$.
[1]Fifteen-second (1,500 mL) tap water spray at 10 psi from a nozzle with an internal diameter of 4 mm.
[2]Sanitizer was based on 200 ppm quaternary ammonium chloride.
[3]Contained 200 ppm sodium hypochlorite.

covered per square; means were calculated, and the data were analyzed using a statistical software package [29].

RESULTS AND DISCUSSION

Results from the first set of experiments (15 s immersion in sanitizer) are shown in Table 1. Water spray without chemical sanitizer caused a significant decrease in the numbers of *Campylobacter*, coliforms, and *E. coli* on the floor squares. However, addition of an immersion dip for 15 s in either sanitizer (quaternary ammonium or sodium hypochlorite) did not significantly improve the removal or inactivation of bacterial cells.

Increasing the sanitizer immersion time revealed an interesting phenomenon. Immersion in the quaternary ammonium based sanitizer for 1 min did make a difference in the numbers of bacteria detected (Table 2). However, the difference was opposite from what was expected. Higher numbers of bacteria were recovered from squares that had been immersed in quaternary ammonium compound than from those that had only been spray washed. This trend was repeatable and resulted in a statistically significant increase in bacterial recovery. The increase was even more pronounced when washed flooring squares were immersed in either sodium hypochlorite or quaternary ammonium based sanitizer for 5 min (Table 3).

These data beg the question of why more *Campylobacter* were recovered from floor squares that had been treated with chemical sanitizers. Spraying with water effectively removed most, but not all, visible gut contents on the squares. Gut contents remaining on water spray control squares were allowed to simply dry for 15 min while that remaining on the experimental squares was remoistened in the sanitizer dip first. This resulted in the small amount of remaining gut contents being noticeably wetter on the experimental squares. All spray-washed floor squares probably had about the same number of viable bacteria after their respective treatment; however, bacteria in

TABLE 2. Mean number of bacteria (log cfu ± 95% confidence interval) recovered from squares (5 × 5 cm) of fiberglass transport coop flooring originally contaminated with 1 g of gut contents then subjected to a 15-s spray of water and a 60-s dip in sanitizer (n = 30)

Treatment	*Campylobacter*	Coliforms	*Escherichia coli*
None (control)	$6.7^{a} \pm 0.2$	$6.1^{a} \pm 0.2$	$5.9^{a} \pm 0.2$
Water wash[1] only	$4.0^{c} \pm 0.5$	$4.4^{c} \pm 0.4$	$4.2^{c} \pm 0.4$
Water wash + sanitizer[2]	$4.7^{b} \pm 0.4$	$5.0^{b} \pm 0.3$	$4.7^{b} \pm 0.3$
Water wash + chlorine[3]	$4.3^{b,c} \pm 0.5$	$4.5^{c} \pm 0.3$	$4.2^{c} \pm 0.3$

[a–c]Values within columns with no like superscripts are significantly different by Tukey's honestly significant difference (HSD) at $P < 0.05$.
[1]Fifteen-second (1,500 mL) tap water spray at 10 psi from a nozzle with internal diameter of 4 mm.
[2]Sanitizer was based on 200 ppm quaternary ammonium chloride.
[3]Contained 200 ppm sodium hypochlorite.

TABLE 3. Mean number of bacteria (log cfu ± 95% confidence interval) recovered from squares (5 × 5 cm) of fiberglass transport coop flooring originally contaminated with 1 g of gut contents then subjected to a 15-s spray of water and a 5-min dip in sanitizer (n = 30)

Treatment	*Campylobacter*	Coliforms	*Escherichia coli*
None (control)	6.4[a] ± 0.3	6.2[a] ± 0.1	5.9[a] ± 0.1
Water wash[1] only	2.3[c] ± 0.4	3.5[d] ± 0.2	3.2[c] ± 0.2
Water wash + sanitizer[2]	3.9[b] ± 0.5	4.3[b] ± 0.3	4.0[b] ± 0.3
Water wash + chlorine[3]	3.4[b] ± 0.6	4.0[c] ± 0.3	3.7[b] ± 0.4

[a–c]Values within columns with no like superscripts are significantly different by Tukey's honestly significant difference (HSD) at $P < 0.05$.
[1]Fifteen-second (1,500 mL) tap water spray at 10 psi from a nozzle with internal diameter of 4 mm.
[2]Sanitizer was based 200 ppm quaternary ammonium chloride.
[3]Contained 200 ppm sodium hypochlorite.

moist gut contents were more readily recovered by the methods used. This means that even a small amount of moist fecal material remaining on the floor of a sanitized coop could result in transfer of *Campylobacter* to broilers.

Ineffective coop washing and sanitizing has been noted before. In a commercial application, Slader et al. [8] found that washed coops still had *Campylobacter* and *Salmonella* when they arrived at the next farm for reuse. Further study of commercial coop washing revealed that the level of sanitizer was not always accurately set resulting in contaminated crates leaving the sanitizing system [16]. We know that this was not the problem in the current study because sanitizers were prepared immediately before use and were not heavily fouled with organic matter.

Higher levels of sanitizer have been reported to be effective to sanitize coops and coop components. Ramesh et al. [14] tested 13 sanitizers against bacteria attached to steel. They found sodium hypochlorite at 250 ppm to be 1 of the 2 most effective sanitizers. Sodium hypochlorite at 500 ppm and 55°C was also found to be effective to decontaminate steel [15]. However, steel is not the most important surface in a transport coop because most of the feces is deposited on the coop floor, which is not usually made of steel. Ramesh et al. [11, 12, 13] studied decontamination of entire transport coops. They found that 1,000 ppm sodium hypochlorite applied at 70°C was an effective sanitizing combination. However, chlorine levels this high are difficult to maintain in a commercial system, especially at high temperatures where objectionable odors and off gassing may become problematic.

A high-pressure spray washer may result in more effective removal of fecal matter than the 10 psi used in the current study. However, personal observation has shown that a standard water hose is often the tool of choice for washing coops at commercial poultry plants. These hoses do not produce the type of pressure that a high-pressure washer can generate. Indeed, washing with high pressure may cause material on the coop surfaces to be spread about through airborne droplets causing other contamination concerns [9].

A possible solution would be to follow the sanitizer application with a second spray wash step to remove small amounts of sanitizer moistened feces. However, that would use twice the amount of water resulting in a more expensive procedure. At the flow rate used in the current study, 1,500 mL of water was used to spray wash each 25 cm^2 piece of flooring. For one full compartment in a dump coop this would translate to about 56,400 mL [30]. Eight hundred and forty six liters would be needed to spray an entire 3-tier, 5-row set of coops; 1,692 L would be required if a second wash was applied after application of sanitizer. The current U.S. national average cost for water (and sewer) is $4.00/1,000 gal [31], which is $1.05 per 1,000 L. Therefore, to spray wash a coop 2 times would cost an average of $1.79 in water costs alone. The economic and environmental cost of water makes this type of coop washing program hard to justify. This is especially so when the literature suggests that

even fully functional commercial coop washing systems fail to remove all pathogenic bacteria from coop surfaces [8, 16]. Clearly, new methods to decontaminate transport coops are needed to effectively sanitize these important surfaces without undue use of natural resources.

CONCLUSIONS AND APPLICATIONS

1. Spraying soiled transport coop flooring with tap water can significantly reduce the number of *Campylobacter*, *E. coli*, and coliforms present.
2. Additional treatment by immersion in sodium hypochlorite or quaternary ammonium sanitizer at 200 ppm is not effective to further reduce numbers of bacteria on coop flooring.
3. Coop washing programs require large amounts of water and can, therefore, be expensive. Such programs need to be examined to ensure that the expense is justified by a documented improvement in coop decontamination.
4. Research is required to uncover new methods to decontaminate broiler transport coops.

REFERENCES AND NOTES

1. Allerberger, F., N. Al Jazrawi, P. Kreidl, M. P. Dierich, G. Feierl, I. Hein, and M. Wagner. 2003. Barbecued chicken causing a multi-state outbreak of *Campylobacter jejuni* enteritis. Infection 31:19–23.

2. Effler, P., M. C. Ieong, A. Kimura, M. Nakata, R. Burr, E. Cremer, and L. Slutsker. 2001. Sporadic *Campylobacter jejuni* infections in Hawaii: Associations with prior antibiotic use and commercially prepared chicken. J. Infect. Dis. 183:1152–1155.

3. Zhao, C., B. Ge, J. De Villena, R. Sudler, E. Yeh, S. Zhao, D. G. White, D. Wagner, and J. Meng. 2001. Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the Greater Washington, DC area. Appl. Environ. Microbiol. 67:5431–5436.

4. Hinton, A., Jr., R. J. Buhr, and K. D. Ingram. 2000. Physical, chemical and microbiological changes in the crop of broiler chickens subjected to incremental feed withdrawal. Poult. Sci. 79:212–218.

5. Byrd, J. A., D. E. Corrier, and M. E. Hume, R. H. Bailey, L. H. Stanker, and B. M. Hargis. 1998. Effect of feed withdrawal on *Campylobacter* in the crops of market age broiler chickens. Avian Dis. 42:802–806.

6. Whyte, P., J. D. Collins, K. McGill, C. Monahan, and H. O'Mahony. 2001. The effect of transportation stress on excretion rates of *Campylobacters* in market-age broilers. Poult. Sci. 80:817–820.

7. Stern, N. J., P. J. Fedorka-Cray, J. S. Bailey, N. A. Cox, S. E. Craven, K. L. Hiett, M. T. Musgrove, S. Ladely, D. E. Cosby, and G. C. Mead. 2001. Distribution of *Campylobacter* spp. in selected US poultry production and processing operations. J. Food Prot. 64:1705–1710.

8. Slader, J., G. Domingue, F. Jorgensen, K. McAlpine, R. J. Owen, F. J. Bolton, and T. J. Humphrey. 2002. Impact of transport crate reuse and of catching and processing on *Campylobacter* and *Salmonella* contamination of broiler chickens. Appl. Environ. Microbiol. 68:713–719.

9. Newell, D. G., J. E. Shreeve, M. Toszeghy, G. Domingue, S. Bull, T. Humphrey, and G. Mead. 2001. Changes in the carriage of *Campylobacter* strains by poultry carcasses during processing in abattoirs. Appl. Environ. Microbiol. 67:2636–2640.

10. Berrang, M. E., J. K. Northcutt, D. L. Fletcher, and N. A. Cox. 2003. Role of dump cage fecal contamination in the transfer of *Campylobacter* to carcasses of previously negative broilers. J. Appl. Poult. Res. 12:190–195.

11. Ramesh, N., S. W. Joseph, L. E. Carr, L. W. Douglass, and F. W. Wheaton. 2003. Serial disinfection with heat and chlorine to reduce microorganism populations on poultry transport containers. J. Food Prot. 66:793–797.

12. Ramesh, N., L. E. Carr, S. W. Joseph, L. W. Douglass, and F. W. Wheaton. 2004. A prototype poultry transport container decontamination system: 1. Design and construction of the system. Tras. ASAE 47:541–547.

13. Ramesh, N., S. W. Joseph, L. E. Carr, L. W. Douglass, and F. W. Wheaton. 2004. A prototype poultry transport container decontamination system: II. Evaluation of cleaning and disinfection efficiency. Trans. ASAE 47:549–556.

14. Ramesh, N., S. W. Joseph, L. E. Carr, L. W. Douglass, and F. W. Wheaton. 2002. Evaluation of chemical disinfectants for the elimination of *Salmonella* biofilms from poultry transport containers. Poult. Sci. 81:904–910.

15. El-Assaad, F. G., L. E. Stewart, E. T. Mallinson, L. E. Carr, S. W. Joseph, and G. E. Berney. 1995. Decontamination of poultry transport cages. Trans. ASAE 38:1213–1220.

16. Corry, J. E. L., V. M. Allen, W. R. Hudson, M. F. Breslin, and R. H. Davies. 2002. Sources of *Salmonella* on broiler carcasses during transportation and processing: modes of contamination and methods of control. J. Appl. Microbiol. 92:424–432.

17. Ramabu, S. S., N. S. Boxall, P. Madie, and S. G. Fenwick. 2004. Some potential sources for transmission of *Campylobacter* jejuni to broiler chickens. Lett. Appl. Microbiol. 39:252–256.

18. Northcutt, J. K., and D. R. Jones. 2004. A survey of water use and common industry practices in commercial broiler processing facilities. J. Appl. Poult. Res. 13:48–54.

19. Stern, N. J., B. Wojton, and K. Kwiatek. 1992. A differential selective medium and dry ice generated atmosphere for recovery of *Campylobacter jejuni*. J. Food Prot. 55:514–517.

20. Campy cefex plates incubated at 42°C for 48 h in a resealable bag that had been flushed with a microaerobic gas mix: 5% O_2, 10% CO_2, and 85% N_2.

21. Spectronic 20D+, Spectronic Instruments, Rochester, NY 14625.

22. RQS Sanitizer, R-Square Products, Gainesville, GA.

23. Clorox Company, Oakland, CA.

24. Self-filling vials containing N,N-diethyl-p-phenylenediamine (Chlorine 2 vacu-vials) were used with Single Analyte Meter (SAM) Chlorine 2 model I-2001, Chemetrics, Calverton, VA.

25. Becton Dickinson Microbiology Systems, Sparks, MD.

26. Petrifilm, 3M Microbiology Products, St. Paul, MN.

27. Microgen Products Limited, Camberley, UK.

28. Petrifilm *E. coli*/coliform count plates were incubated at 35°C for 24 h, aerobically.

29. Within each set of experiments the data were handled as a randomized complete block design with replication as the blocking factor. An analysis of variance was performed to determine if a treatment effect existed. Comparison of individual treatments was done using Tukey's honest significant difference test. Significance was set at $P < 0.05$. Statistica, StatSoft, Tulsa, OK.

30. 0.94 m^2 per compartment or 940 cm^2, 940/25 = 37.6, 37.6 × 1,500 = 56,400.

31. Northcutt, J. K. 2002. Conserving water and washdown effectiveness. Pages 73–75 in Proc. Poult. Processors Workshop. US Poult. Egg Assoc., Atlanta, GA.

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